Bone Imaging and Mechanical Testing

WU Musculoskeletal Research Center
Musculoskeletal Structure &Strength Core

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June 20, 2014

Washington University in St. Louis

Musculoskeletal Research Center at Washington University

Questions

- Does my mouse have a skeletal phenotype?
- I treated rats with drug XYZ … what was the effect on their bones?
- What outcomes do I need to measure?
- How do I integrate microCT and mechanical testing outcomes?

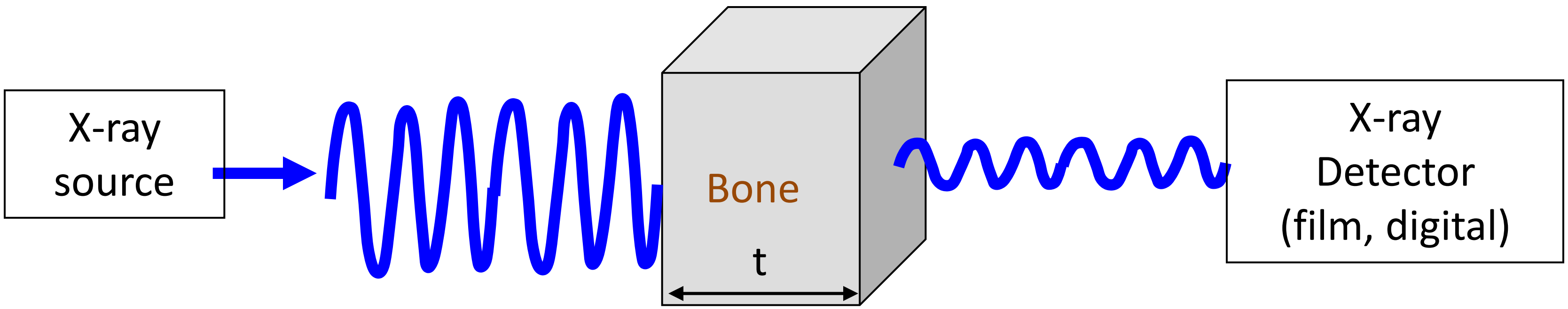
Structure & Strength

- Imaging → Structure
  - how much bone is there? how is it distributed?
  - density, morphology (geometry)
  - whole-bone, cortical, cancellous
- Mechanical Testing → Strength
  - stiffness, strength, toughness
  - length scale
    - big: 'structural' (whole-bone, organ)
    - small: 'material' (tissue)

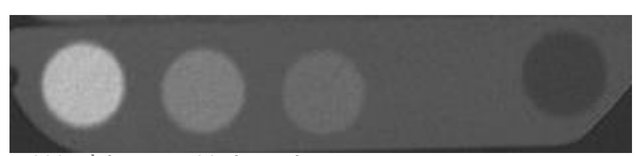
Imaging → Structure

X-Ray Based Imaging

- Based on x-ray absorption/attenuation



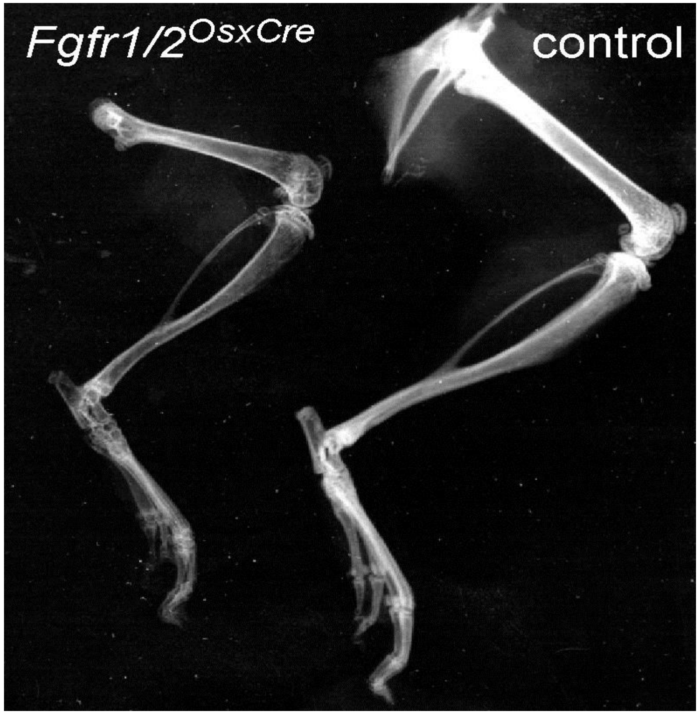
- Attenuation depends on thickness (t), density
- Can calibrate x-ray absorption to:
  - Equivalent density of hydroxyapatite (HA) phantom



Radiography

Faxitron

- Outcomes: qualitative phenotype, spontaneous fractures, fracture callus



Kai Yu, Dave Ornitz

2 week post-fracture

10 week

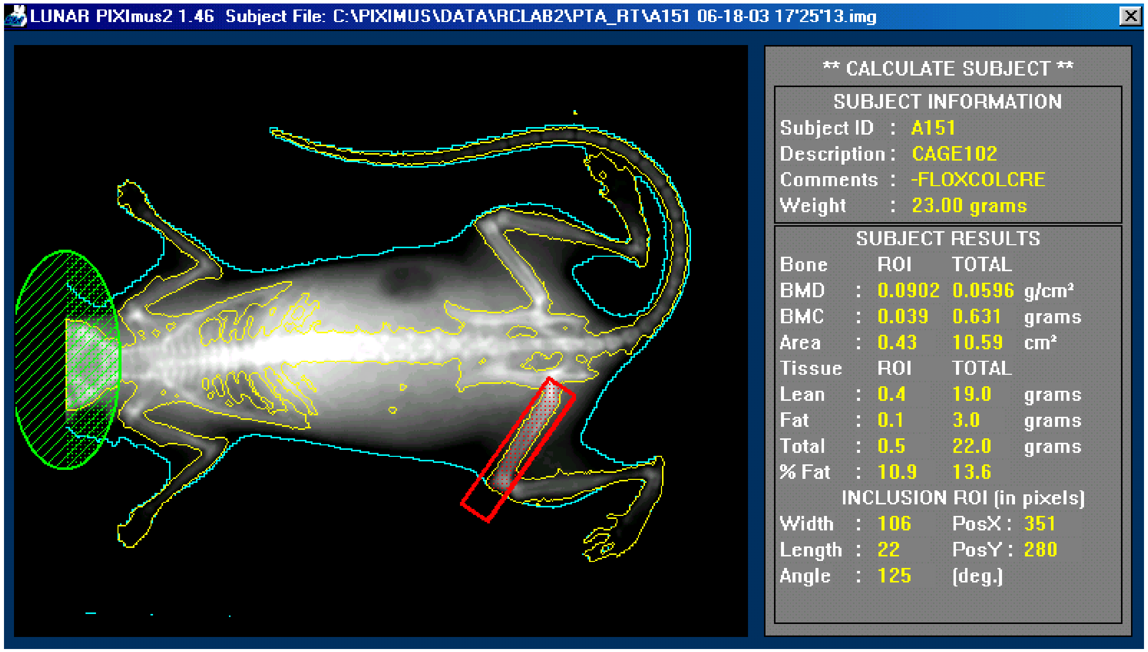
5 month

18 month

Jenny McKenzie, Evan Buettmann, Mike Gardner

DXA

GE/Lunar Piximus



Piximus

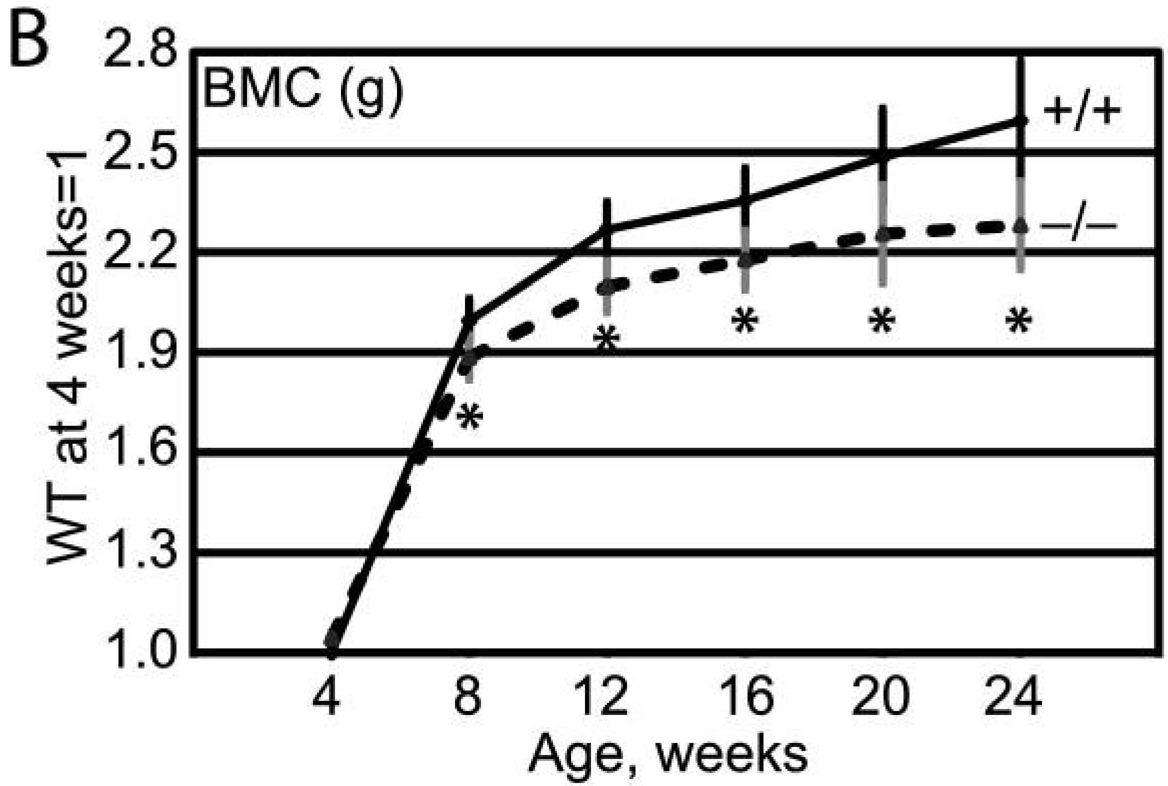
__Outcomes__:

- BMC (g), aBMD (g/cm^2), %fat

__Uses__:

- Whole animal
- Phenotyping
- Systemic intervention
- Longitudinal studies

DXA shows decreased post-natal bone mass in MAGP-deficient mice

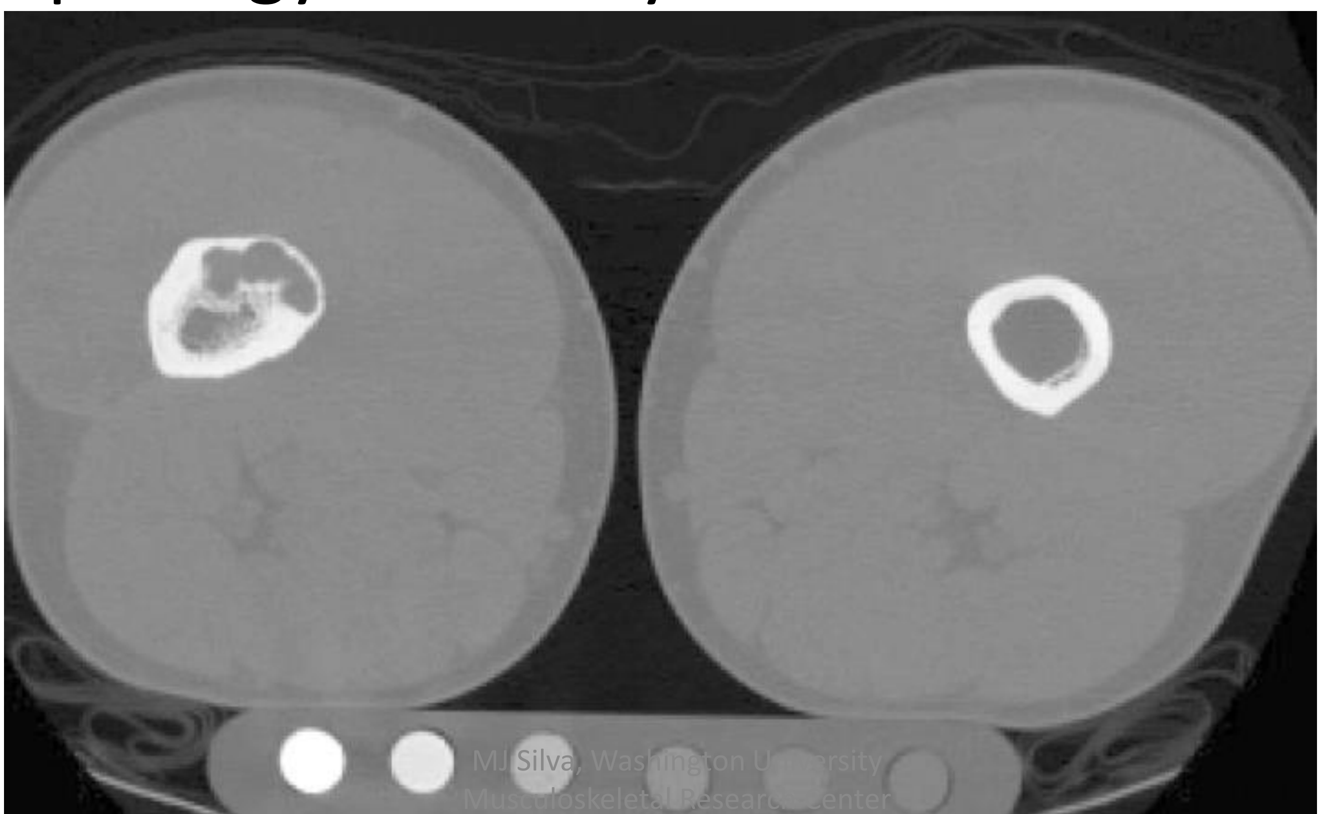


- Phenotyping
- Longitudinal
- Whole animal

Craft, Mecham et al., 2010

QCT – Quantitative Computed Tomography

- grayscale image reflects local attenuation, i.e., “mineral density map” [whiter = denser]
- morphology & density



microCT

REVIEW

JBMR

Guidelines for Assessment of Bone Microstructure in Rodents Using Micro–Computed Tomography

Mary L Bouxsein,[1] Stephen K Boyd,[2] Blaine A Christiansen,[1] Robert E Guldberg,[3]
Karl J Jepsen,[4] and Ralph Müller[5]

Journal of Bone and Mineral Research, Vol. 25, No. 7, July 2010, pp 1468–1486

microCT – regions of interest

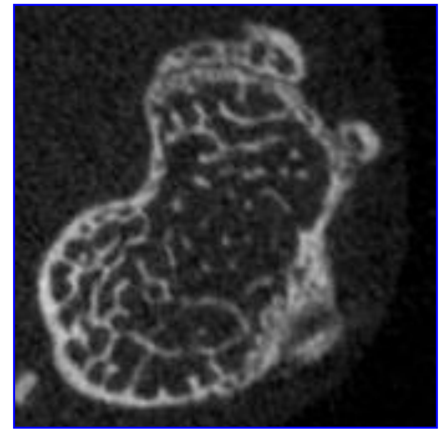
Cancellous/Trabecular/Metaphyseal

Scan region

- 100 slices = 2 mm

Analysis region

- landmark (eg, growth plate)
- 40 slices = 0.8 mm



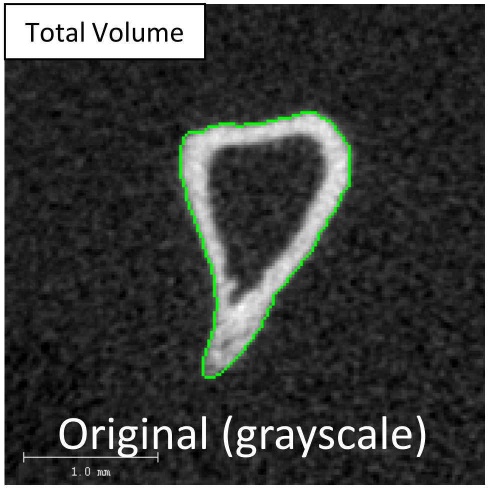
Cortical/Diaphyseal

Scan region = Analysis region

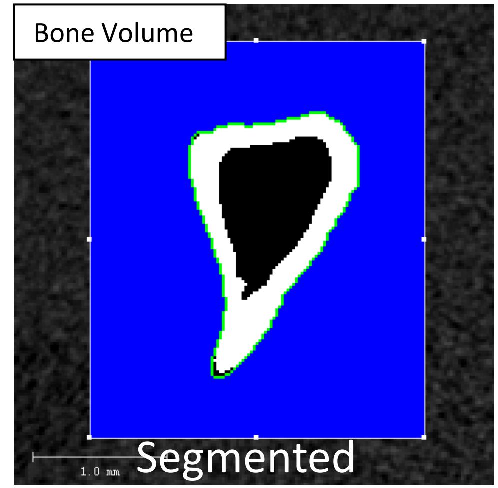
- Mid-point (50% of length), or X mm from TFJ
- 35 slices = 0.7 mm

microCT – cortical analysis

Contour



Threshold/
Segment



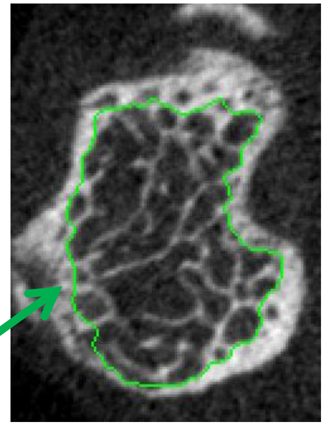
Binary
- white=bone
- black=not bone

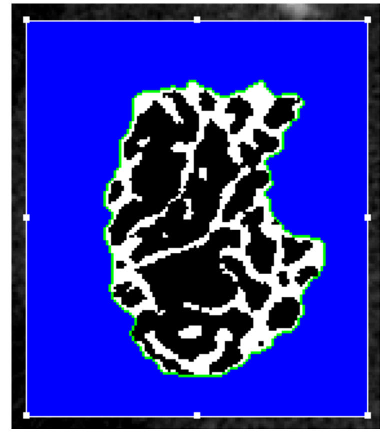
<u>Cortical Outcomes</u>

- Total area (or volume) – How big is the bone? → periosteal apposition
- Bone (cortical) area – How much bone is there? → formation & resorption
- Marrow area → resorption
- Cortical thickness → formation & resorption
- Area moment of inertia → How is bone distributed? Resists bending loads.
- Tissue mineral density (“mean2”) → mineralization

microCT – cancellous analysis

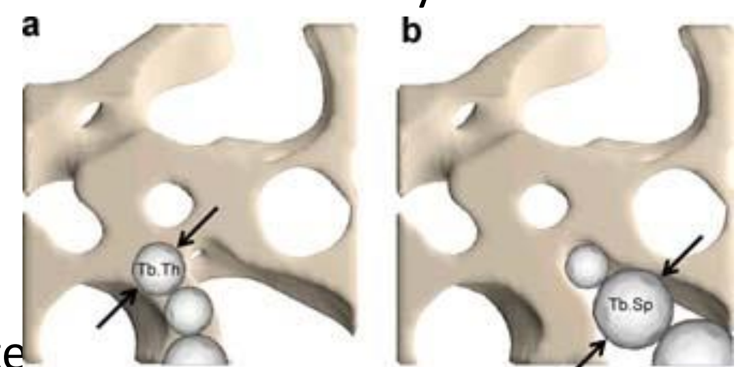
Total volume (TV)





Bone volume (BV)

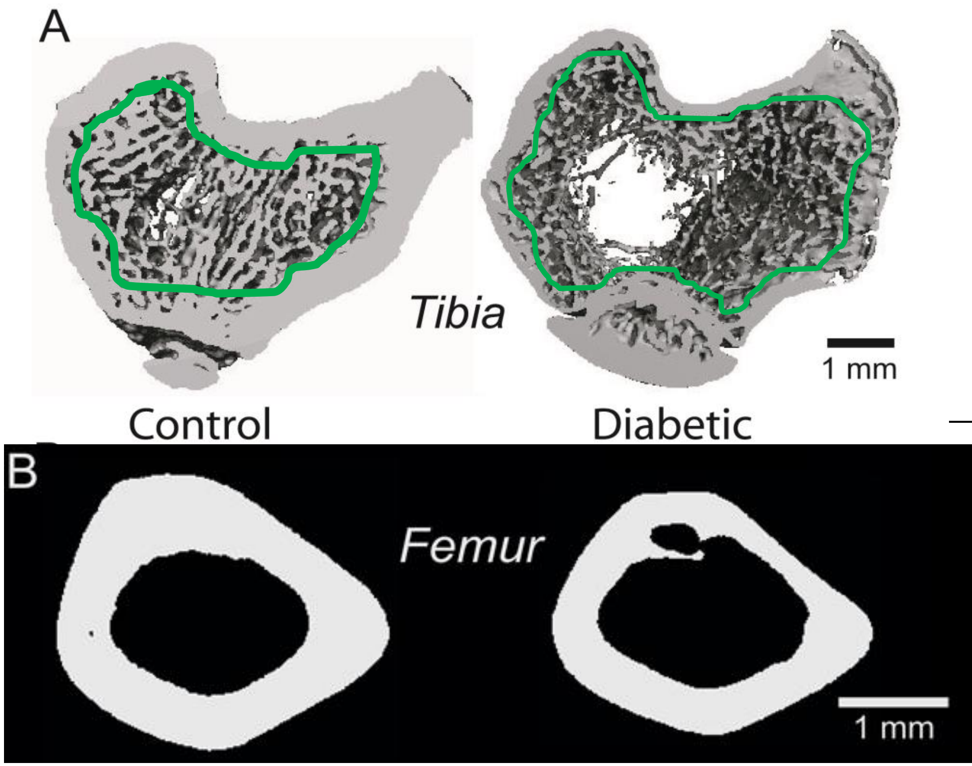
- <u>Cancellous Outcomes</u>*
  - Bone volume fraction (BV/TV) – What fraction of marrow cavity is filled with bone?
  - Trabecular thickness (Tb.Th)
  - Separation (Tb.Sp)
  - Trabecular Number (Tb.N)
  - Density, vBMD (“mean1”) – Should correlate



Bouxsein et al. 2010

* Use Direct Method values; prefix “VOX”; do not use Plate Model values; prefix “TRI”

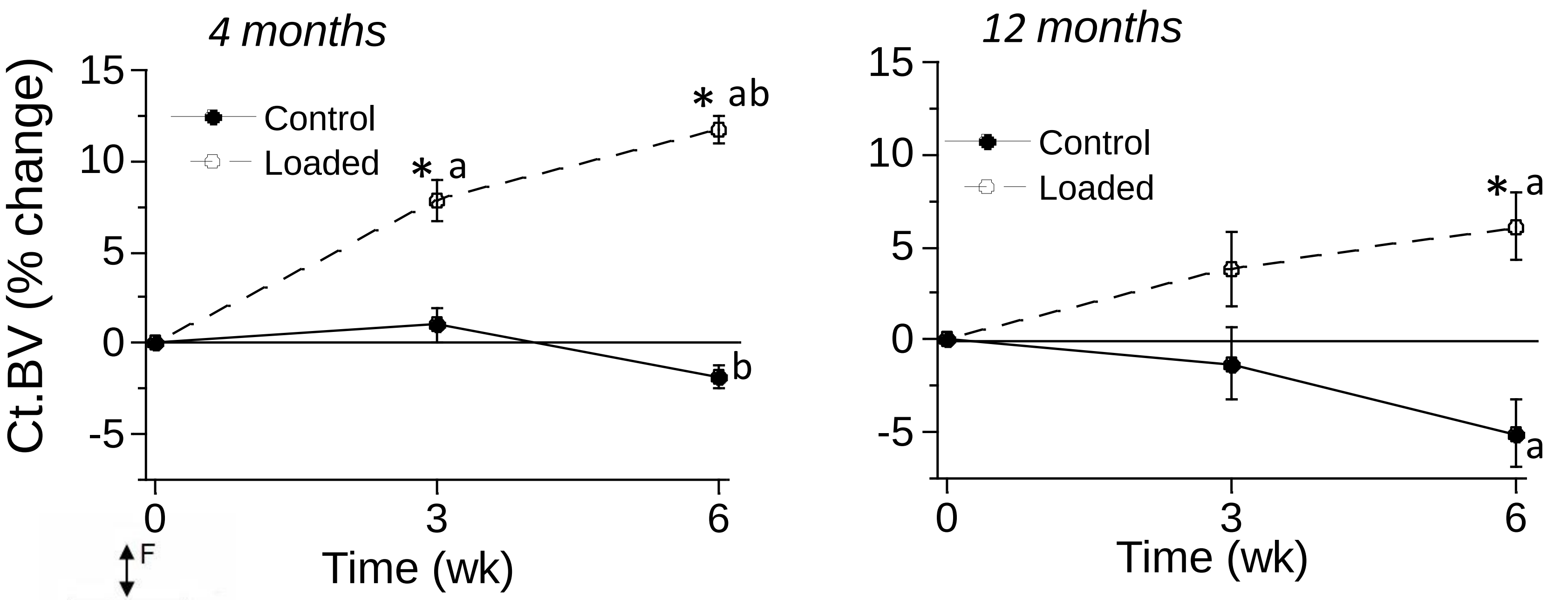
Specimen microCT reveals osteopenia in diabetic rats

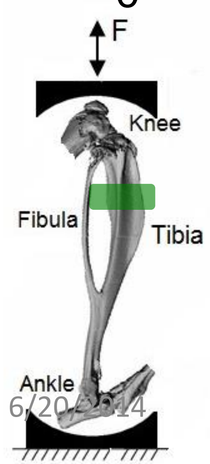


	Parameter	Control (n = 9)	Diabetic (n = 8)
Trabecular	Tb.BV/TV (mm^3/mm^3)	0.37 ± 0.04	0.12 * ± 0.04
Trabecular	Tb.Th (mm)	0.11 ± 0.01	0.07 * ± 0.01
Trabecular	Tb.N (1/mm)	4.4 ± 0.2	2.6 * ± 0.5
Cortical	Bone Area (mm^2)	5.85 ± 0.30	4.46 * ± 0.27
Cortical	Moment of Inertia (mm^4)	4.03 ± 0.89	3.03 * ± 0.54
Cortical	Cortical TMD (mg/cm^3)	1092 ± 33	1111 ± 51

Silva et al. 2009

In Vivo CT: Mechanical Loading Stimulates Cortical Bone Accrual (in Age-Dependent Manner)





BALB/c
female mice

Silva et al., 2012

Mechanical Testing → Strength

How to measure bone mechanical properties

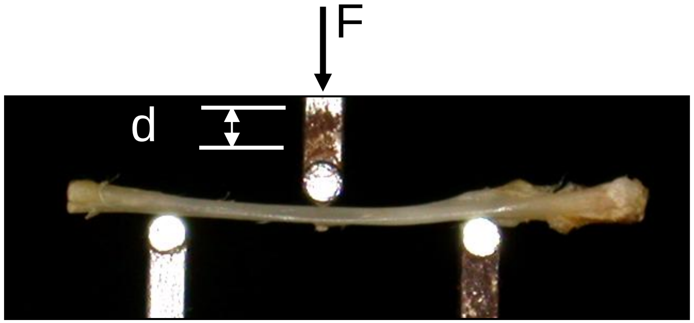
- No

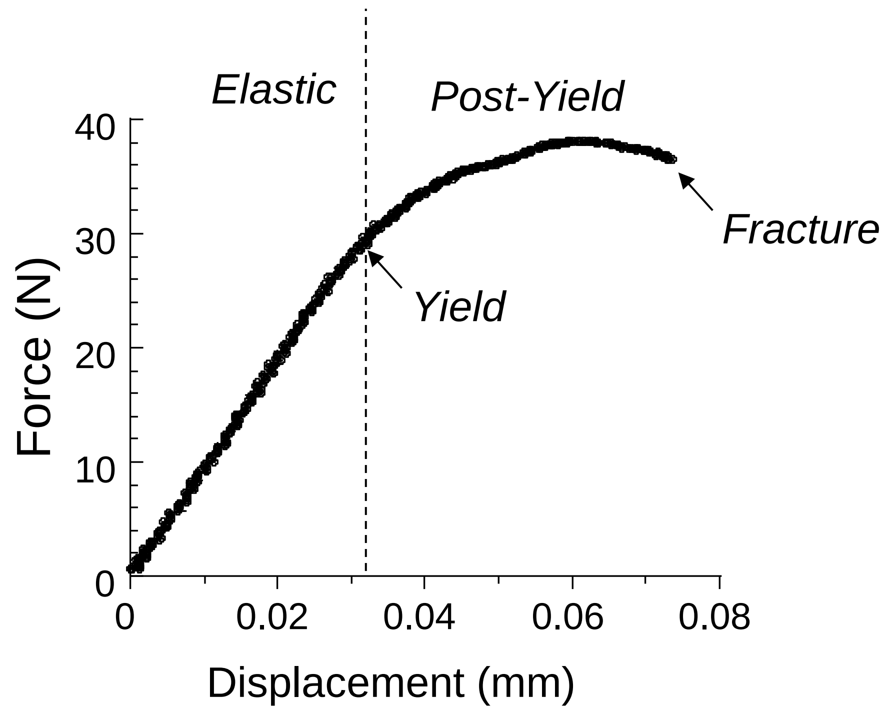
- Yes

Instron mechanical testing machine

Mechanical Testing: Force-Displacement Plot

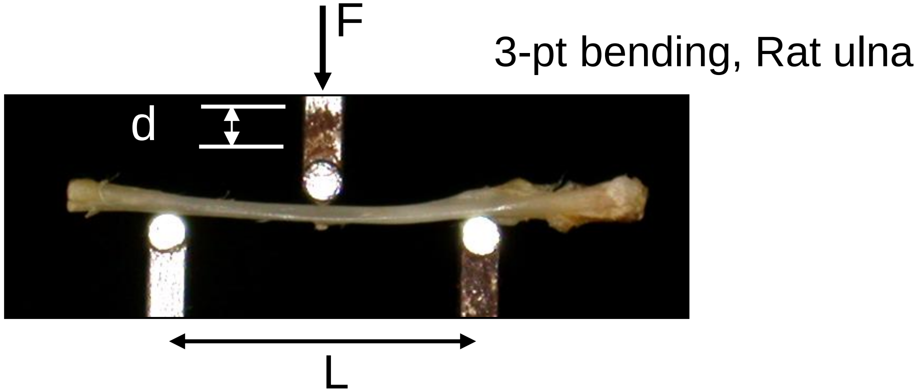
3-pt bending, Rat ulna

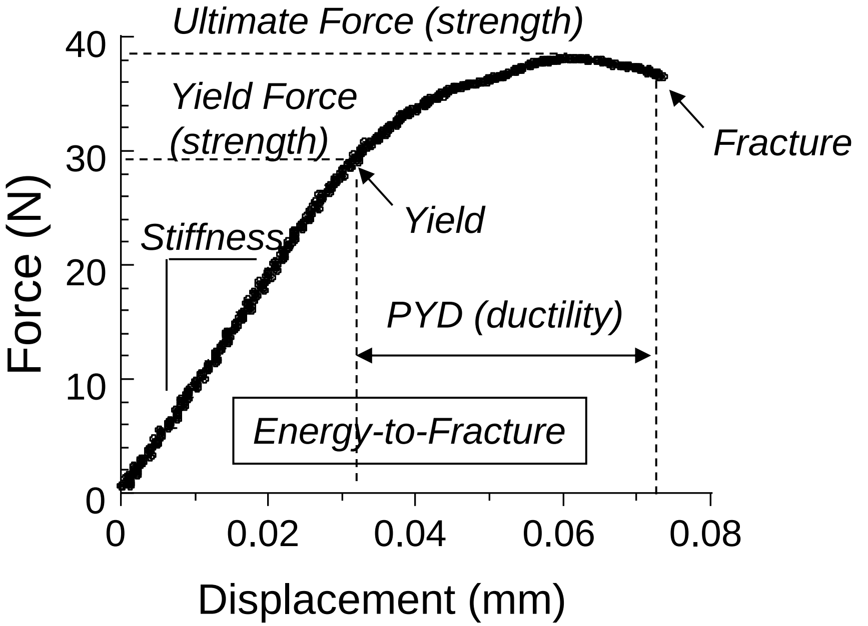




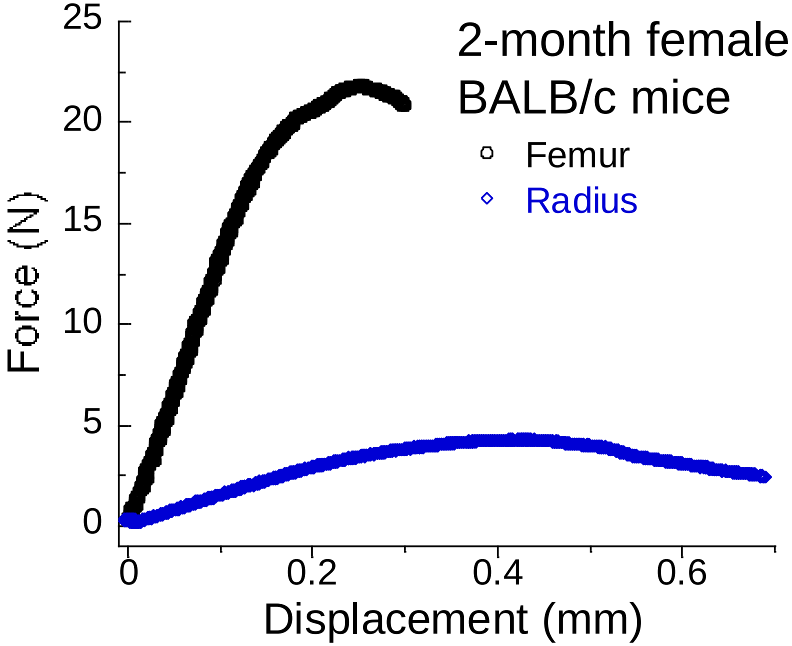
Mechanical Properties

- Stiffness (N/mm)
- Strength
  - Yield Force (N)
  - Ultimate Force (N)
- Post-yield displacement (ductility) (mm)
- Energy-to-fracture (Nmm)





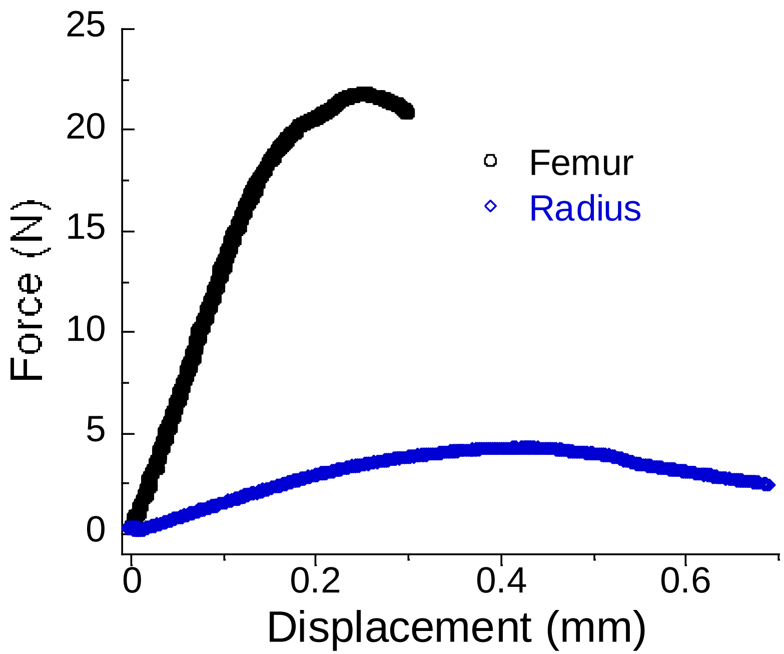
Mechanical properties

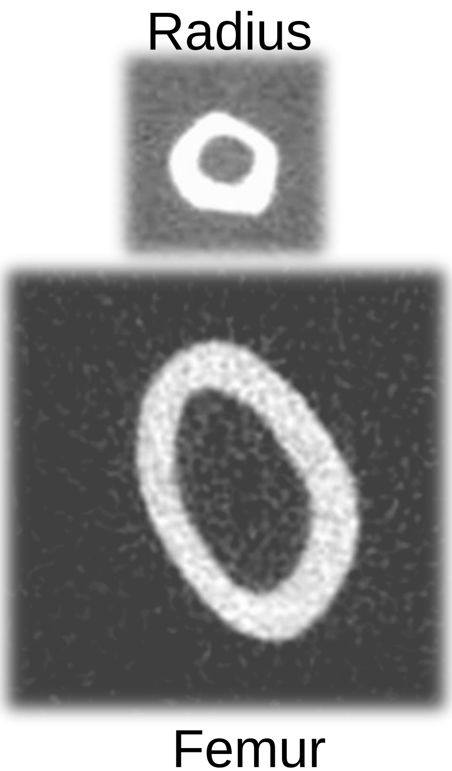


Femur vs. Radius:

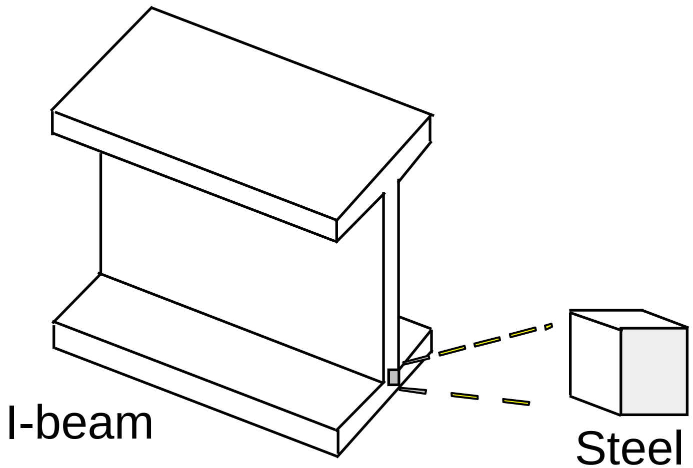
- stiffer
- stronger
- less ductile
- requires more energy to fracture

Whole-bone properties depend on <u>morphology</u> & <u>material</u>



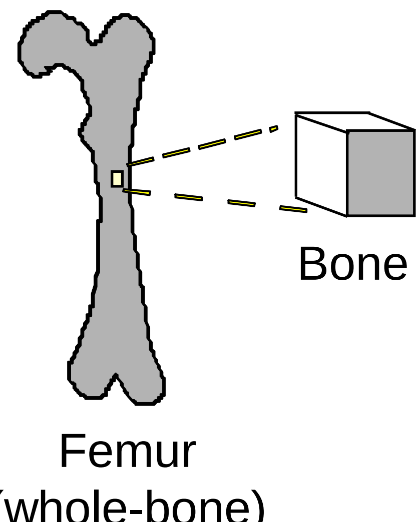


MJ Silva, Washington University
Musculoskeletal Research Center



Big:
- Structure
- Whole-Bone

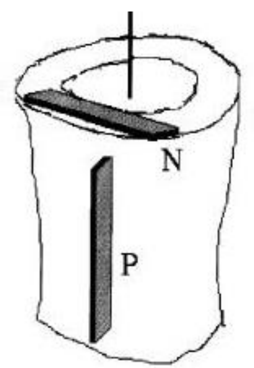
Small:
- Material
- Tissue



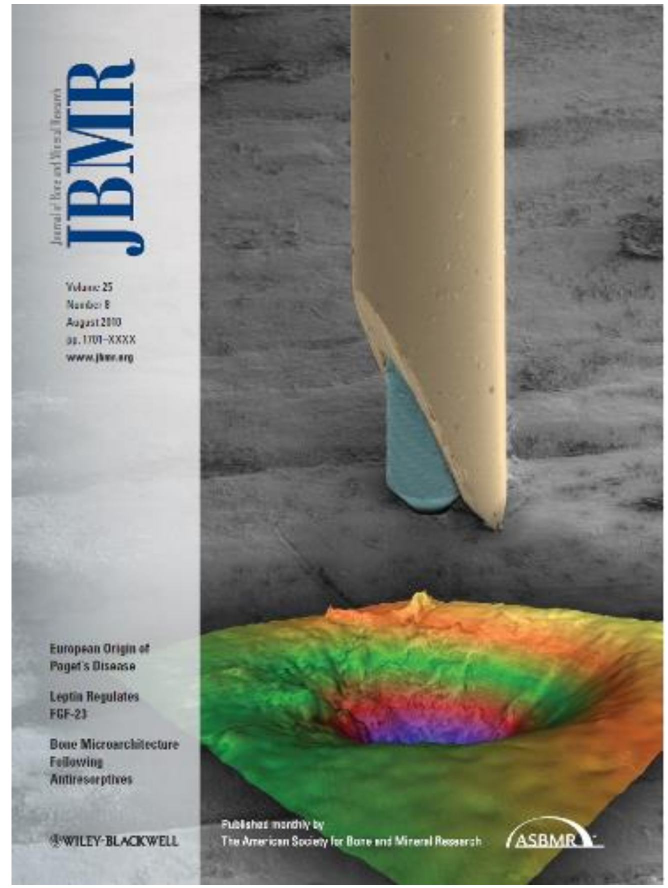
Material Properties

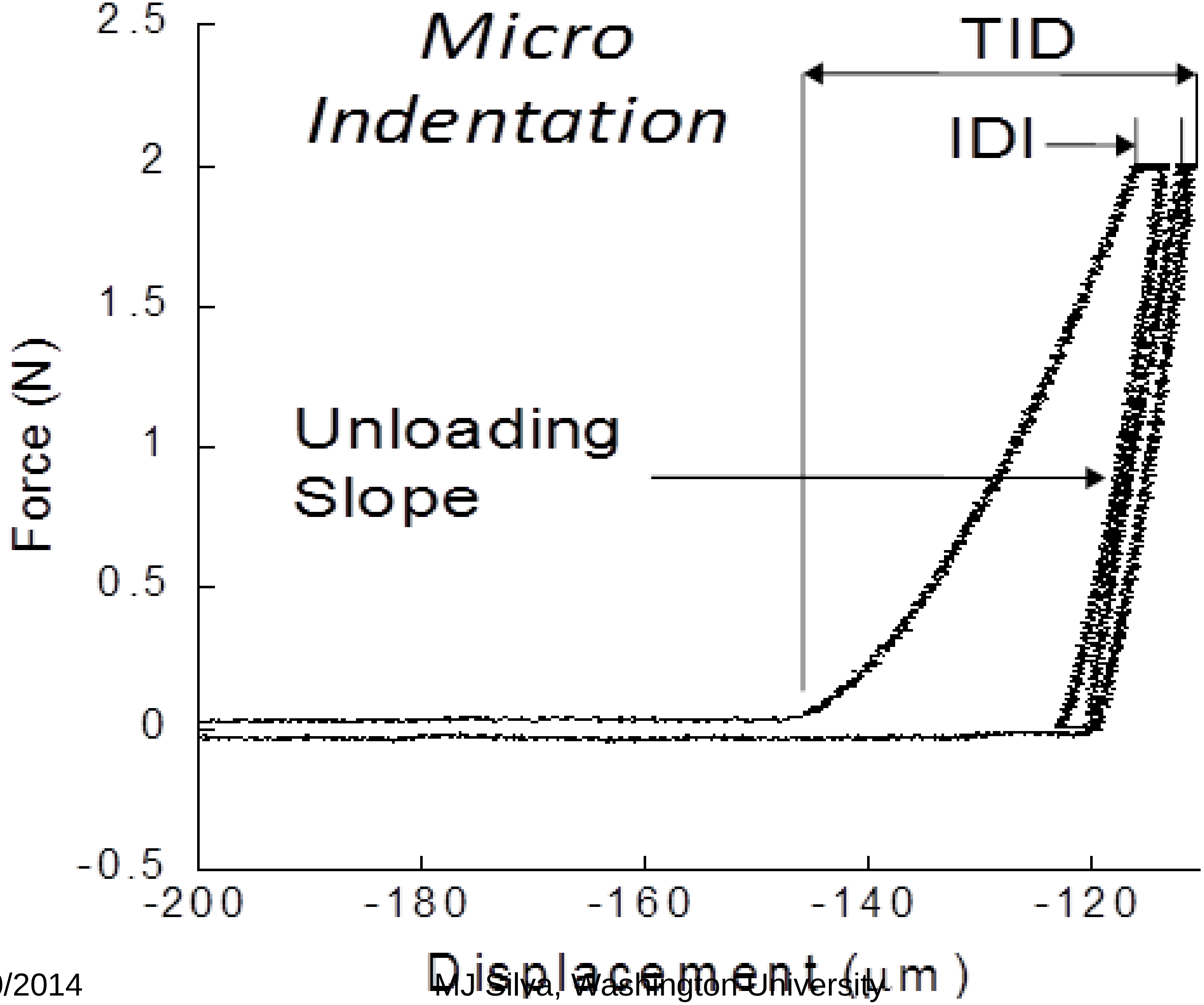
- Estimate from whole-bone mechanical test and bone size (microCT)
  - Whole-bone strength: Ultimate Force (N)
  - Material strength: Ultimate Stress (N/mm^2)

- Measure with tissue-level test
  - small piece of bone
  - microindentation



Microindentation

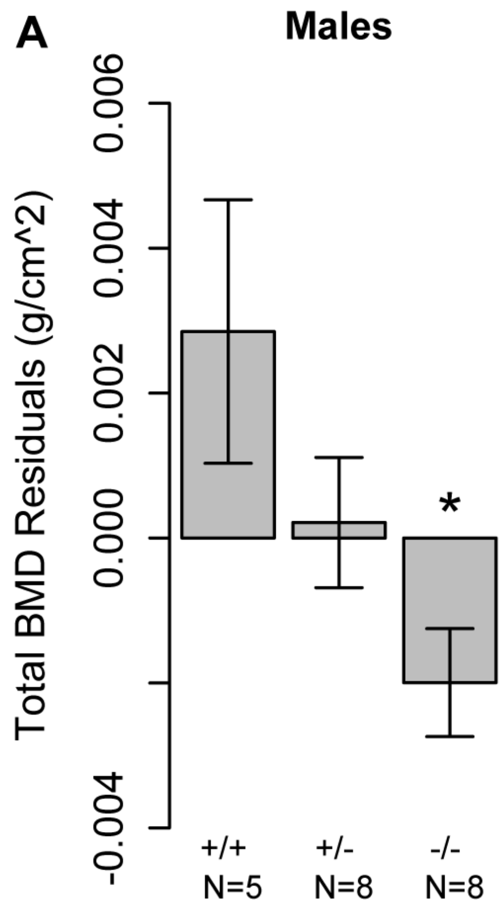



Micro Indentation
TID
IDI
Unloading Slope
Force (N)
2.5
2
1.5
1
0.5
0
-0.5
-200
-180
-160
-140
-120
Displacement (μm)

Case Study 1: Asxl2 null mice

Wei Zou, Steve Teitelbaum

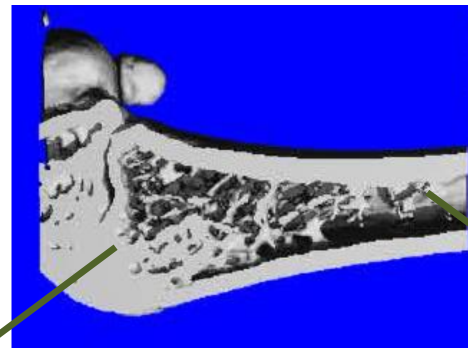
Asxl2-null mice have low BMD



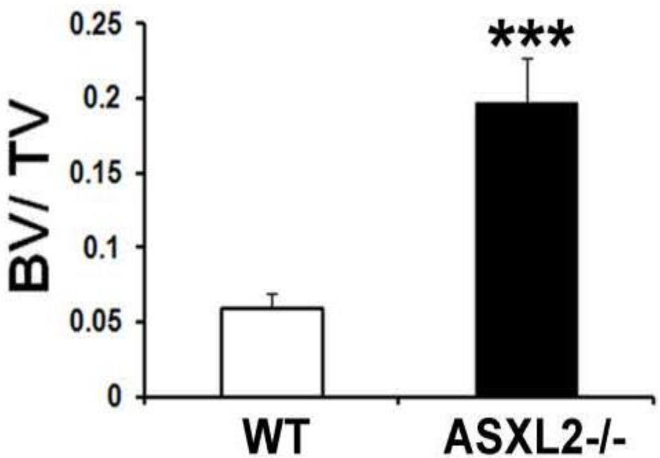
age- and body weight-adjusted

Farber et al, 2011

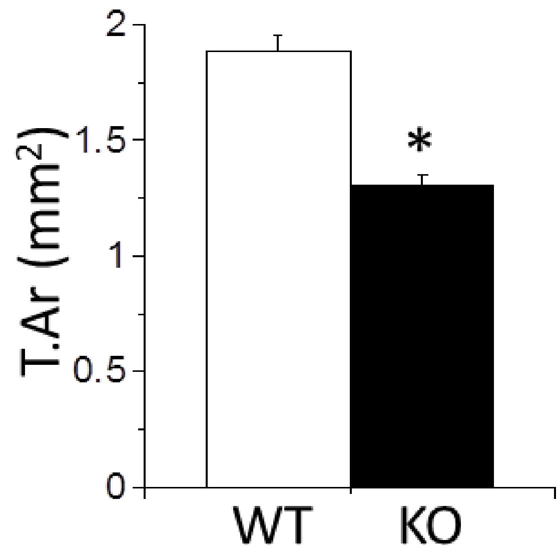
Asxl2-null mice: more cancellous bone, but less cortical bone



Cancellous



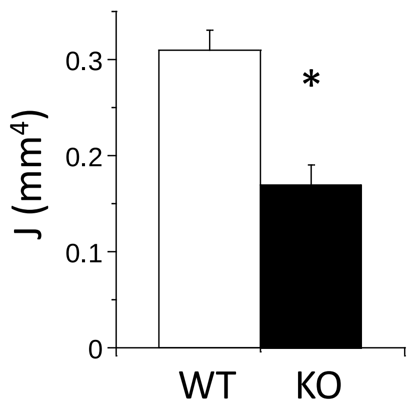
Cortical

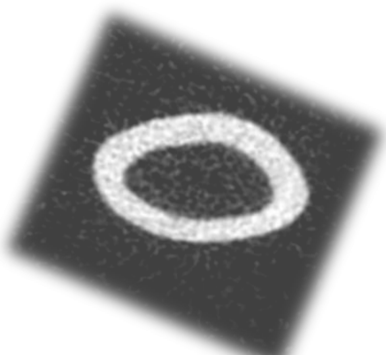


Wei Zou

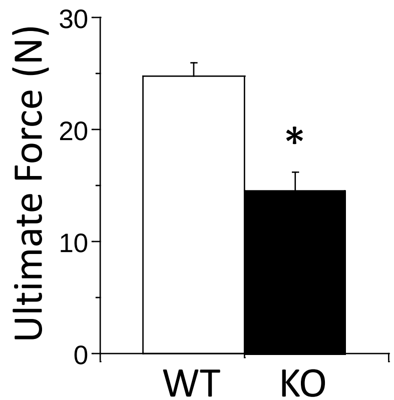
Asxl2-null femurs: small & weak bones, normal material strength

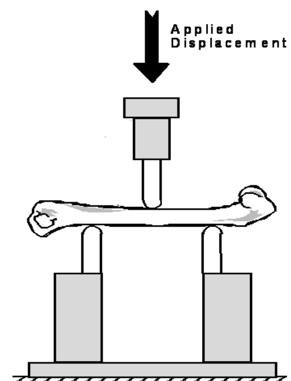
Moment of Inertia



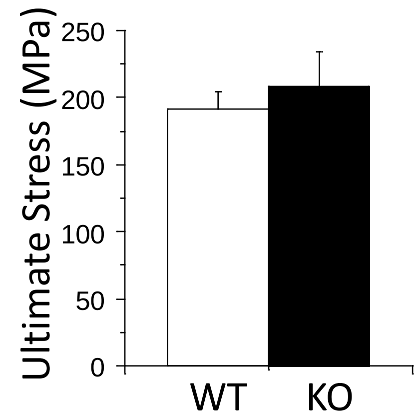


Whole-bone strength





Material strength



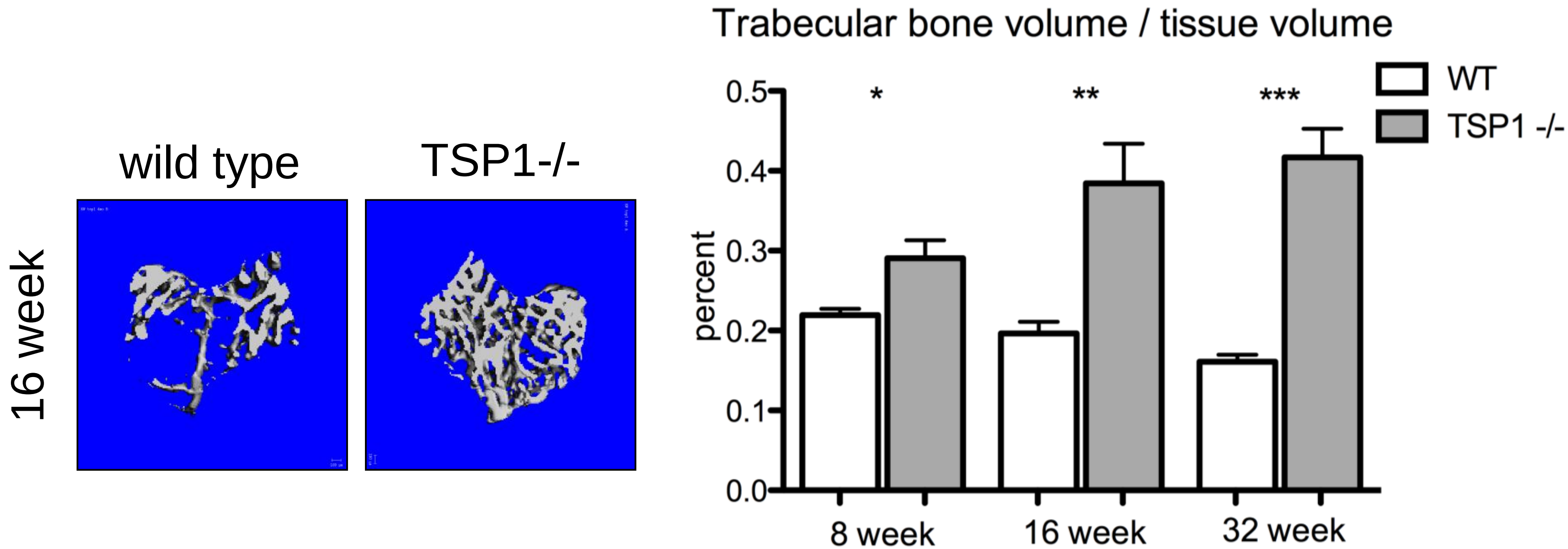
Asxl2-null mice - Summary

- Increased trabecular bone (osteoclast defect)
- Decreased bone size & whole-body BMD
- Bones are weaker in proportion to their smaller size
- Normal material properties

Case Study 2: Tsp1 null mice

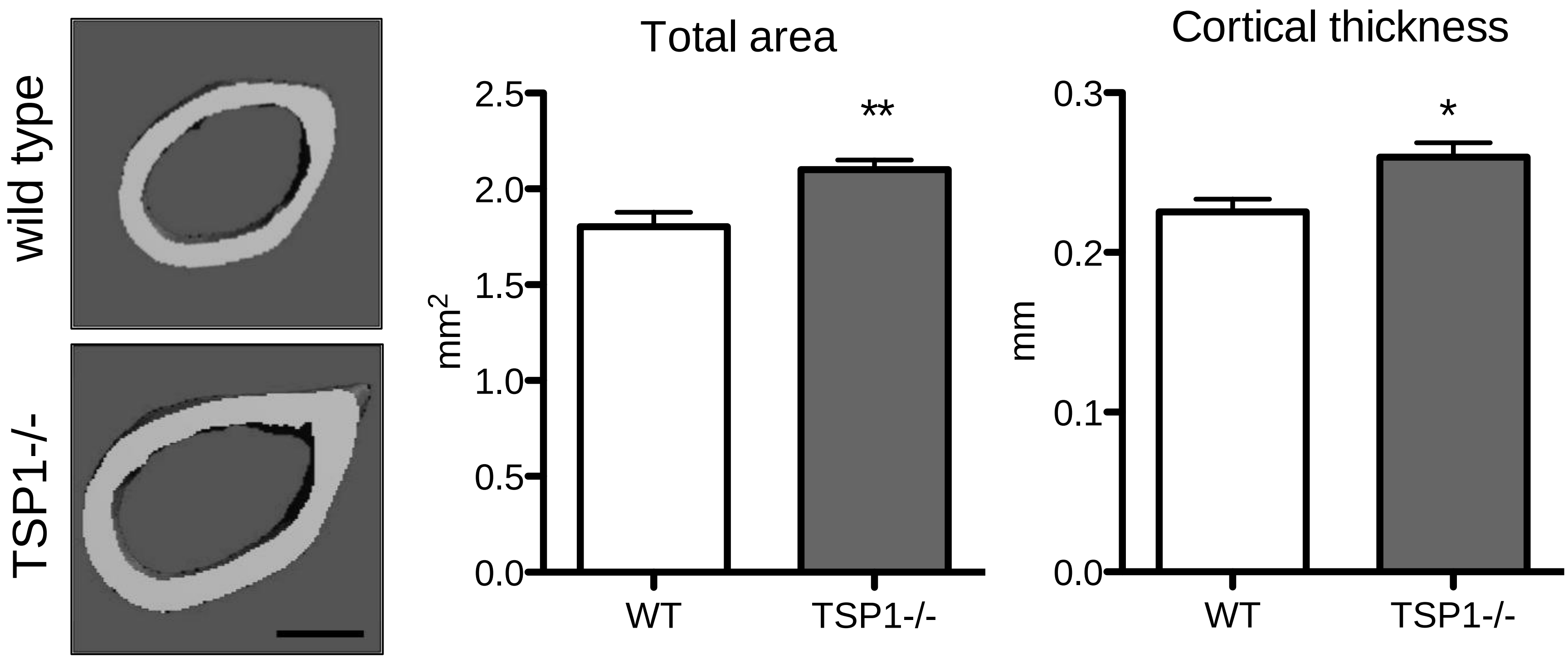
Sarah Amend, Kathy Weilbaecher

TSP1-/- mice have increased trabecular bone



Amend et al., In review

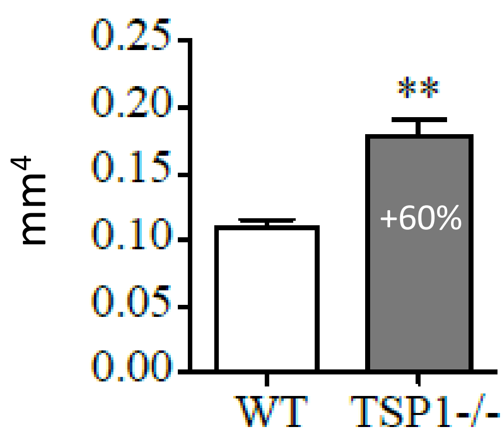
TSP1-/- mice have larger bones



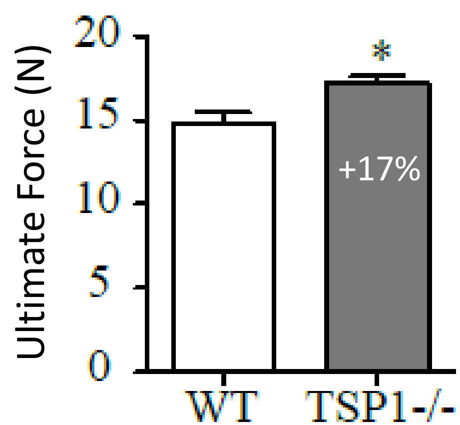
Amend et al., In review

Tsp1-null femurs: larger, moderately stronger, weaker material

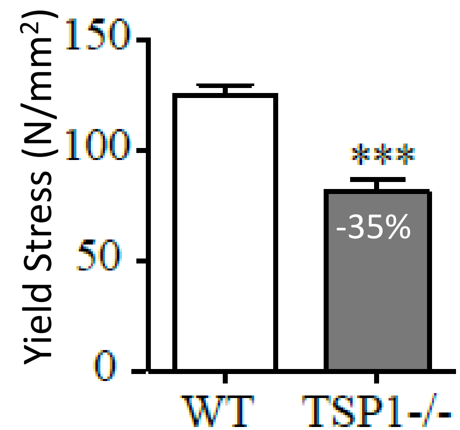
Moment of Inertia

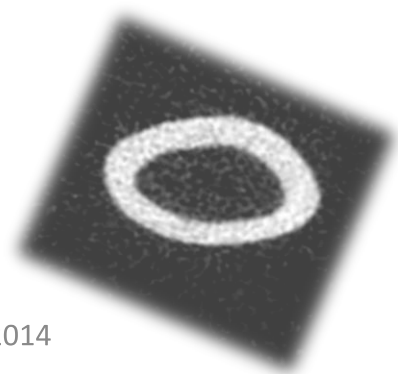


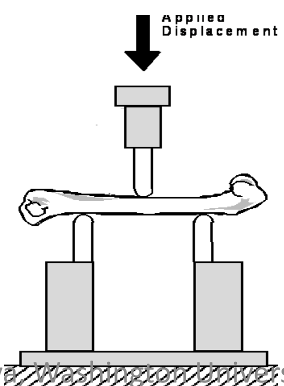
Whole-bone strength



Material strength

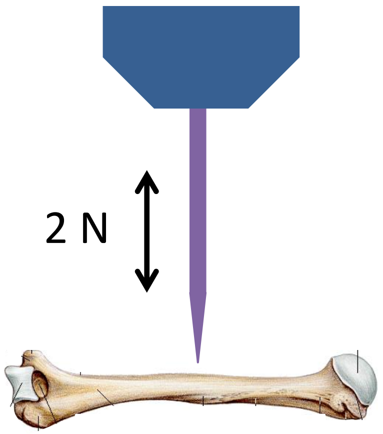




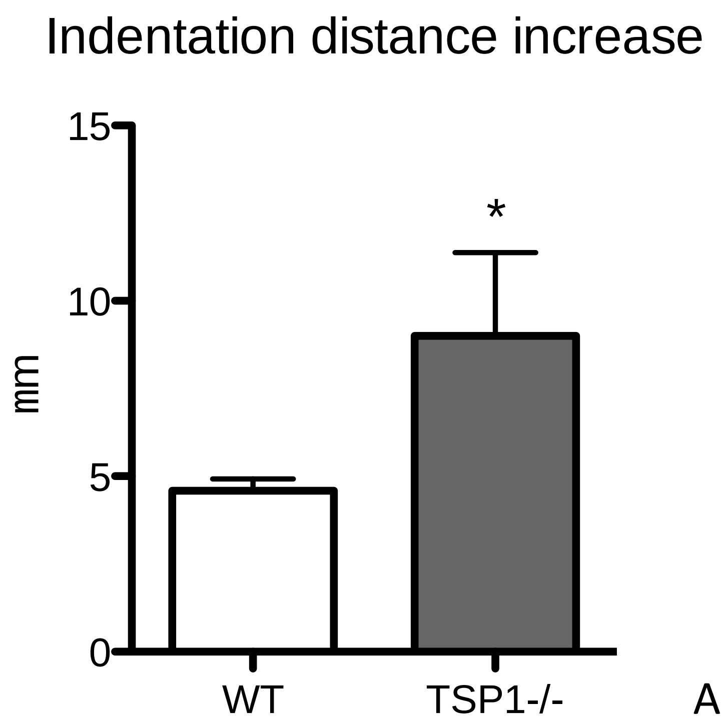


Amend et al., In review

TSP1-null bone: damages more easily at material level



microindentation



Amend et al., In review

TSP1-null mice: Summary

- Increased trabecular bone
- Increased cortical bone size
- Bones are moderately stronger – disproportionate to size
- Impaired material properties

musculoskeletalcore.wustl.edu

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DEPARTMENT OF ORTHOPAEDIC SURGERY
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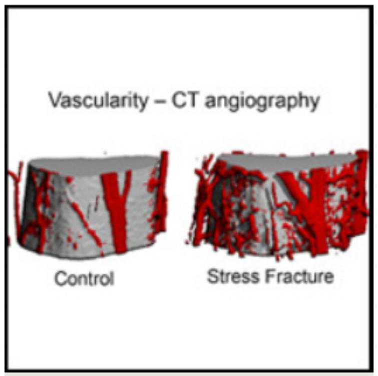

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Overview

Structure and strength are perhaps the most relevant properties when assessing functional outcomes in animal models related to musculoskeletal biology and medicine. The **objectives** of the **Musculoskeletal Structure and Strength Core** are: 1) to increase access to existing resources for densitometry, imaging, and mechanical testing; 2) to enhance expert oversight and quality control; 3) to provide training and enrichment opportunities related to core services; and 4) to foster new interactions and enhance existing interactions between members of the Research Base of the Center for Musculoskeletal Research at Washington University.

Consultation: Contact the Core Director or any of our Associate Directors or Technical Staff to discuss your project. We can advise you on outcomes you may consider for a musculoskeletal structure / biomechanics experiment, and what we can do to help you obtain these outcomes. If you are in the planning stages or submitting a proposal, we can help with study design a priori. If you are starting your experiment, we can provide training or do the work for you. If you have collected your data, we can help with data analysis and interpretation.

Imaging: We support the use of x-ray based imaging of musculoskeletal structures and tissues from animal models (mouse to canine) generated by Research Base investigators. Available techniques include plane radiography, dual-energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and micro-computed tomography (microCT). Importantly, each of these techniques is available for in vivo as well as post mortem imaging.

Mechanical Testing: We perform mechanical testing to assess the functional

SERVICES
- Overview
- Fee Schedule and Forms
- Technical Notes
- Core B News
- Project Gallery
- Publications
- Image Library

Acknowledgements

- Dan Leib
- Michael Brodt
- Tarpit Patel
- Steve Thomopoulos
- Simon Tang

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NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases